Acta vet. scand. 1975, *16*, 477—479.

Brief Communication

DEOXYRIBONUCLEASE PRODUCTION BY LEPTOSPIRES

Deoxyribonuclease (DNase) production has been examined for many microorganisms and its significance has been related to pathogenicity and taxonomy. Enzymatic studies on leptospires have previously been concentrated on lipase activity. Recently, however, *Kadis & Pugh* (1974) proved that pathogenic strains of L. grippotyphosa and L. icterohaemorrhagiae exhibited urease activity. The present communication reports on DNase production by 10 strains of six different serotypes*. The strains are listed in legend to Fig. 1.

The organisms were propagated on liquid Tween 80 Albumin medium (TAM) (*Ellinghausen & McCullough* 1965) and Korthof's medium (KM) for 24 days at 28°C. The cultures were, prior to investigation of DNase activity by agar diffusion and spectrophotometrical methods, controlled both on solid and liquid medium that no DNase producing organisms except the leptospires were present.

The cultures were centrifuged for 10 min. (20000 $\times$ g), and the supernatants were applied into circular wells of 7 mm diameter in a toluidine blue deoxyribonucleic acid agar (TDA) (*Lachica et al.* 1971) which, prior to application, was poured into glass trays to a depth of 2 mm. The TDA agar was modified by substituting 0.01 M-$CaCl_2$ with 0.01 M-$MgSO_4$, and the pH was adjusted to 5.6 with 0.1 M acetate buffer. As controls culture supernatants in TDA without DNA and uninoculated medium in TDA were used. The TDA medium was incubated at 37°C for 20 hrs. Fig. 1 presents the DNase activities of the strains cultivated on TAM. Positive reaction occurred as pink circular zones in the otherwise blue medium. Only one strain was negative. A semi-quantitative measure of DNase activity was obtained by

* In this communication the nomenclature of the 7th edition of Bergey's Manual is used.

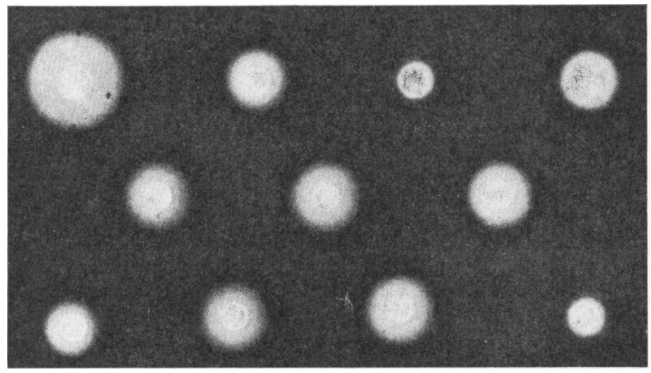

Figure 1. Pink circular zones indicating DNase activity of the strains investigated. The strains are from upper left to lower right: No. 1 L. canicola, No. 2 L. sejroe, No. 3 L. grippotyphosa, No. 4 L. pomona, No. 5 L. pomona, No. 6 L. pyrogenes, No. 7 L. pomona, No. 8 L. pomona, No. 9 L. australis, No. 10 L. canicola. Well No. 11 is filled with uninoculated medium.

determining the number of diffusion units in the supernatant according to *Sandvik* (1962) and *Dahle* (1969). Table 1 shows the enzyme activity (diffusion units) of the strains. For spectrophotometrial examination 0.5 ml of the supernatant of strain No. 1 was added to parallel tubes, each with 1 ml substrate* and 1 ml 0.1 M acetate buffer, pH 5.6 and incubated at 37°C in a water bath. The reaction was interrupted at 20 and 30 min. by adding 1 ml 5 % trichloracetic acid followed by centrifugation (20000 × g) for 15 min. The absorbance of the supernatants

Table 1. Number of diffusion units of the strains investigated.

Strain no. (see Fig. 1)	Diff. units	Strain	Diff. units
1	2240	6	200
2	70	7	125
3	0	8	16
4	70	9	125
5	125	10	125

* 150 mg DNA (Difco)
50 ml 0.01 M-$MgSO_4$
50 ml aqua dest.

was measured at 260 nm. The absorbance at 20 and 30 min. were 0.580 and 1.075, respectively. Uninoculated KM was used as reference.

In conclusion it has been shown that with one exception, the leptospires examined produce DNases when cultivated in liquid media. The DNase production was measured both by agar diffusion and spectrophotometrical methods. The importance of the DNA splitting activity of leptospires is uncertain. However, it seems justified to discuss the presence of this tissue destroying enzyme in relation to pathogenicity. This prospect should be subjected to further research.

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(Received July 16, 1975).

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